UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
DEPARTAMENTO DE FARMÁCIA

KLÉCIA SANTOS DOS ANJOS

HOST-GUEST INCLUSION COMPLEX CONTAINING β-CYCLODEXTRIN AND (-)-MYRTENOL MODULATE HYPERALGESIA, ANXIETY, COGNITIVE ALTERATIONS AND REDUCE OXIDATIVE STRESS IN A FIBROMYALGIA-LIKE MODEL

SÃO CRISTÓVÃO, SE
2018

KLÉCIA SANTOS DOS ANJOS

HOST-GUEST INCLUSION COMPLEX CONTAINING β-CYCLODEXTRIN AND (-)-MYRTENOL MODULATE HYPERALGESIA, ANXIETY, COGNITIVE ALTERATIONS AND REDUCE OXIDATIVE STRESS IN A FIBROMYALGIA-LIKE MODEL

Trabalho de Conclusão de Curso apresentado à Coordenação do Curso de Farmácia do Centro de Ciências Biológicas e da Saúde da Universidade Federal de Sergipe, como requisito parcial para obtenção do título de Bacharel em Farmácia.

Orientador: Prof. Dr. Lucindo José Quintans Júnior

SÃO CRISTÓVÃO, SE

2018

KLÉCIA SANTOS DOS ANJOS

HOST-GUEST INCLUSION COMPLEX CONTAINING β-CYCLODEXTRIN AND (-)-MYRTENOL MODULATE HYPERALGESIA, ANXIETY, COGNITIVE ALTERATIONS AND REDUCE OXIDATIVE STRESS IN A FIBROMYALGIA-LIKE MODEL

Aprovada em: __/__/____

Banca Examinadora

Professor Dr. Lucindo José Quintans Júnior (Orientador)

Professor Dr. Marcelo Cavalcante Duarte (DFA/UFS-Examinador Interno)

Professora Dra. Adriana Gibara Guimarães (Examinadora Externa)

“É exatamente disso que a vida é feita, de momentos. Momentos que temos que passar, sendo bons ou ruins, para o nosso próprio aprendizado. Nunca esquecendo do mais importante: Nada nessa vida é por acaso. Absolutamente nada. Por isso, temos que nos preocupar em fazer a nossa parte, da melhor forma possível. A vida nem sempre segue a nossa vontade, mas ela é perfeita naquilo que tem que ser.”

Chico Xavier

Agradecimentos

TCC- Trabalho de conclusão de curso. Nos últimos meses venho pensando sobre a importância de terminar ciclos e ficar feliz com isso. Feliz a ponto de curtir o momento e por um instante não pensar no depois. Não pensar nas novas metas ou nos novos desafios a superar. Agora, só busco agradecer a Deus e à toda proteção que me permitiu chegar até aqui, quero curtir esse fim, olhar para trás e saber do caminho que trilhei e tantas mudanças até aqui, tantas evoluções, desafios superados ou fracassados, felicidades, tristezas, crises psicológicas, dúvidas construídas e certezas descontruídas.

Neste momento, quero agradecer ao grupo LANEF (Laboratório de Neurociências e Ensaios Farmacológicos), lugar em que tive a oportunidade de colaborar durante os últimos 4 anos e me ensinou muito do que sei sobre o mundo científico. Agradeço, em especial, a meu orientador, Professor Lucindo, por toda atenção, dedicação, por acreditar em meu potencial, pelas cobranças, sinceridade e, principalmente, por me permitir errar. Parabéns pelo pesquisador que és, ‘’Big boss’’.

Agradeço à Professora Jullyanna por todas as nossas conversas, pela paciência, dedicação e atenção com que lidera esse grupo. E também a todos colaboradores, em especial à Luana por ter trazido tantas novas visões sobre o fazer ciência, pela disponibilidade e atenção; à Alexandra por ouvir meus dramas, pela disponibilidade e ser uma amiga; à Marlange pelas várias contribuições e carinho em cada conversa; à Priscila por ter me ensinado muito sobre organização, compromisso e sobre escrever materiais científicos; a todos os ‘’Lanef The Troopers’’ pelas várias vivências e crescimento juntos (em especial a Janara, Erik, Juliana, Alexandra, Bruno, Jéssica (*in memoriam*), Tainá e Marília). Não poderia esquecer de Thalita, sempre muito atenciosa e carinhosa comigo.

Por fim, mas não por ordem de importância, quero agradecer a minha mãe por toda paciência, por não deixar eu desistir do caminho, por transmitir força e amor. Quero também agradecer e pedir desculpa a minha vó e minha tia Anita pelas minhas ausências, pelas vezes que não deu para ficar junto. Muito obrigada, família. Aos meus amigos, obrigada por não desistir de mim nunca. Quero agradecer, principalmente, à Cácia, Jéssica, Drielly, Janaina, Isabella e Marianna. Amo vocês.

Muito obrigada!

Klécia S.

Host-guest inclusion complex containing β-cyclodextrin and (-)-myrtenol modulates hyperalgesia, anxiety, cognitive alterations and reduce oxidative stress in a mice fibromyalgia-like model

Klécia Santos dos Anjos[1], Alexandra Maria dos Santos Carvalho[1], Luana Heimfarth[1], Bruno Lucena dos Santos[1], Yasmim Maria Barbosa Gomes de Carvalho[2], Antônio Guilherme de Carvalho Neto[2], Mairim Russo Serafin[2], Adriano Antunes de Souza Araújo[2], José Ivo Araújo Beserra Filho[3], Sara Pereira da Silva[3], Alessandra Mussi Ribeiro[3], Daniel Pereira Bezerra[4], Flavio Machado de Souza Carvalho[5], Lucindo José Quintans Júnior[1], Jullyana de Souza Siqueira Quintans[1*]

[1]Department of Physiology, Federal University of Sergipe, São Cristóvão, SE, Brazil.
[2]Department of Pharmacy, Federal University of Sergipe, São Cristóvão, SE, Brazil.
[3]Department of Biosciences, Federal University of São Paulo/UNIFESP, Santos, SP, Brazil.
[4]Oswaldo Cruz Foundation, Laboratory of Tissue Engineering and Immunopharmacology, Salvador, BA, Brazil.
[5]Institute of Geosciences, University of São Paulo, SP, Brazil.

Abbreviations: CNS, Central Nervous System; FM, fibromyalgia; MYR, Myrtenol; βCD, β-cyclodextrin; NPs, natural products; PM, physical mixture; KN, paste complexation; SC, slurry complexation; PGB, Pregabalin.

∗ Corresponding authors at: Federal University of Sergipe. Department of Physiology, Laboratory of Neuroscience and Pharmacological Assays (To JSSQ).
Ave Marechal Rondon, sn, Rosa Elze, São Cristóvão, Sergipe, CEP: 49.100-000, Brazil.
E-mail: jullyanaquintans@gmail.com (Prof J.S.S. Quintans).

Abstract

We evaluated anti-hyperalgesic, anxiolytic and antioxidant activity of the complex containing myrtenol (MYR) and β-ciclodextrin (βCD) in fibromyalgia model (FM). The MYR/βCD complex was prepared and structurally characterized. Male Swiss mice were pretreated with MYR (50 mg/kg; p.o.), MYR/βCD (50 mg/kg; p.o.), pregabalin (PGB, 30 mg/kg; i.p) or vehicle. 1h after of treatment, the mechanical hyperalgesia, grip strength test, open field (OF), novel object recognition test (NOR), elevated plus-maze test (EPM) and some oxidative damage markers were assessed. The characterization tests indicated that MYR was incorporated into βCD. MYR/βCD had anti-hyperalgesic effect in all days of treatment and keep this activity during 6 h ($p < 0.001$). No difference was observed in muscle strength and OF test in MYR/βCD compared with negative control ($p > 0.05$). In OR test, the group MYR/ βCD spent more time in novel object to the familiar object ($p < 0.05$). MYR/βCD increase exploration time on open arm ($p < 0.05$) in EPM. MYR/βCD decrease malondialdehyde amount ($p < 0.05$), increased catalase activity ($p < 0.05$) and decreased SOD/CAT ratio when compared to control ($p < 0.05$). MYR/βCD promotes an anti-hyperalgesia effect for 6 h, revealed anxiolytic-like effect, inhibited cognitive and motor impairment and reduce oxidative stress.

Keywords: chronic pain. fibromyalgia. anxiety. monoterpene. cyclodextrin. oxidative Stress.

1. Introduction

Fibromyalgia (FM) is a rheumatic syndrome with a spectrum of symptoms, including generalized chronic pain, excessive tiredness, sleep and mood, cognitive impairment, loss of life quality of life [1, 2]. FM is present around 2% of the world population, especially women; this already means economic costs estimated at more than 12,993 million euros per year in Spain [3, 4]. This syndrome involves multiple symptoms and comorbidities, consequently the ideal treatment remains undefined, requiring both pharmacological and non-pharmacological approaches, based on the control of the individual symptoms, mainly pain [5].

Patients with FM are exposed to oxidative stress process and despite being non-inflammatory rheumatic syndrome there are cytokines such IL-6, IL-8 [6] and IL-10 found altered [7]. Free radicals generated by oxidative stress result in lipid peroxidation and consequently can produce tissue damage. Several clinical evidences support that oxidative stress can also cause peripheral and central sensitization and produce changes in the nociceptive sensitivity threshold, producing hyperalgesia mediated by both local and spinal oxidant mechanisms[8]. Furthermore, the increase in oxidative stress parameters are more strongly associated with severity of FM [9].

The effectiveness of current drugs to control the FM is limited and controversial, with patients discontinuing use due to low clinical efficacy and severe side-effects. Thus, the challenge is the development of new therapeutic proposals effective for the management of FM and others 'dysfunctional pain'[10, 11]. The natural products (NPs), such as terpenes and essential oils, has been shown to be a promising and intriguing source of potential bioactive molecules for novel drug models capable to block or mitigate the FM symptoms [12, 13, 14, 15].

Myrtenol (MYR), an alcohol monoterpene and secondary plant metabolite of the genus *Taxus*, seems to be a promising NP for treatment of FM symptoms because previous studies have shown therapeutic potential, such as anxiolytic [16], gastroprotective [17], anti-inflammatory and antinociceptive properties [18]. Moreover, MYR restored the impairment of endogenous antioxidant enzymes activities against myocardial ischemia-reperfusion injury model [19]. However, it has never been evaluated in chronic non-inflammatory muscular pain induced by acid saline (reported to be a FM animal model), as well as if it could ameliorate others symptoms common in FM patients which the current treatment is relapsing into solving or improving poorly.

Controversially, some NPs, including essential oils and terpenes, have physical properties that depending on the pharmaceutical formulation may become problematic in biological administration, mainly due the poor water solubility and fleeting bioavailability that produces non-durable effects [20, 21]. These physical-chemical properties may also affect the bioavailability and therapeutic effect of the substance [22]. Therefore, the incorporation of these products into macrocyclic hosts, such as cyclodextrins (CDs), have been shown to be favorable being exploited in products that are already available in the pharmaceutical market and in new drug patents [21, 23, 24].

CDs are cyclic oligosaccharide nanoparticles which provide a host system for organic substances since they have an internal hydrophobic cavity available to form non-covalent inclusion complexes with a wide variety of organic molecules of appropriate shapes and sizes [25, 26, 27, 28]. This interaction that occurs with the CD molecules promotes a protection to the complexed substance, so preventing oxidation and protecting from changes in temperature, humidity and even evaporation [21, 23]. This interaction becomes a powder soluble in water and easy to handle, facilitating its insertion into the pharmaceutical [29, 30]. Moreover, MYR similar to other terpenes appears to be a guest promise to be complexed into CD [21, 31].

Thus, we aimed to evaluate the anti-hyperalgesic, emotionality and cognitive effects of MYR using a chronic musculoskeletal pain model in mice (a rodent fibromyalgia-like model), and possible action mechanism of MYR in the oxidative stress process.

2. Material and Methods

2.1. Drugs

β-cyclodextrin (βCD, ≥ 97% purity), (-)-myrtenol (MYR, ≥ 96% purity), epinephrine and catalase were purchased from Sigma-Aldrich (St. Louis, MO, USA), ketamine and xylazin were purchased from Cristália (Itabira, SP, Brazil),

4.1. Preparation of samples

The samples were prepared by techniques of physical mixture (PM), paste complexation (KN) and slurry complexation (SC) as described by Andrade et al. (2017).

In the PM method, MYR (152.23 g/mol) and βCD (1134.98 g/mol) were mechanically mixed by 10 min in the molar ratio of 1:1 in ambient temperature. For to prepare the PC, the MYR and βCD were mixed and adding then 2.0 mL of distilled water with constant manual

stirring until forming a paste and removed by manual trituration. Then, the material was kept in a desiccator until dry. Finally, SC was carried out by the addition of water (20 mL) to a beaker containing MYR and βCD remained under constant magnetic stirring at 150 rpm for 36 h (Quimis Q 261A21, Brazil). Subsequently, the material was stored in the desiccator until dry and removed by manual trituration [32].

4.2. Thermal analysis

Samples were characterized by differential scanning calorimetry (DSC) and thermogravimetry/derivate thermogravimetry (TG/DTG) as described by Andrade et al. [29].

DSC curves were obtained using a DSC50 cell (Shimadzu, Japan) using a heating rate of 10 °C/min from 25 to 500 °C under dynamic nitrogen atmosphere (50 mL/min) in aluminum capsules (Al) containing ~2 mg of samples. The DSC cell was calibrated with indium (melting point 156.6 °C; ΔH fus = 28.5 J.g-1) and zinc (melting point 419.6 °C).

TG/DTG curves were obtained with TGA-60 thermobalance (Shimadzu, Japan) in the temperature range from 25–900 °C, under dynamic nitrogen atmosphere (50 mL/min) using platinum capsules containing ~2 mg of samples. The TG/DTG system was verified using calcium oxalate monohydrate ($CaC_2O_4.H_2O$) with 99.99% purity.

4.3. Scanning electron microscopy (SEM)

The βCD, PM, PC and SC samples were analyzed according to Quintans et al. [33].

4.4. X-ray diffraction (XRD)

XDR patters were obtained on Simens, model D5000, as described by Menezes et al. [32].

2.6. Animals

Three-month-old male Swiss mice (40-50g) were housed with free access to food and water, with 6-8 animals in plastic cages (30 x 37 x 16 cm), under conditions of controlled temperature (22 ± 2 ºC) on 12 h light/12 h dark cycle (lights on 06:00h a.m.). All experiments were carried out from 08:00 h a.m. to 05:00 h p.m. Experimental protocols and all procedures were approved by the local ethics committee of the Federal University of Sergipe (protocol CEPA/UFS nr.16/2017) and lo committee on the use of animals of the Federal University of São Paulo (protocol CEUA nr. 2888011216). All efforts were made to minimize animal pain, suffering or discomfort.

2.7. Acid saline-induced chronic muscle pain

Prior to induction of pain, mice were anesthetized with intraperitoneal (i.p.) ketamine (100 mg/kg) plus xylazine (10 mg/kg). Afterwards, 20µL of acid saline (pH = 4) was injected into the left gastrocnemius muscle to induce chronic widespread non-inflammatory muscle pain. After five days, animals again animals were injected with acid saline. In this model for FM, bilateral mechanical hyperalgesia lasting was induced for 4 weeks after the second injection [34].

2.8. Pharmacological treatments

After confirming mechanical hyperalgesia, mice were treated with MYR (50 mg/kg; p.o.), MYR/βCD (50 mg/kg; p.o.) or vehicle (saline 0.9%; p.o.) daily for 10 days; or pregabalin (PGB, 30 mg/kg, i.p.). After 1 h, animals were evaluated to behavioral tests.

2.9. Mechanical hyperalgesia measurement

Mechanical hyperalgesia was evaluated in mice as reported by Nascimento et al. [12]. In a quiet room, the animals were placed in acrylic cubicles (12×10×17 cm) with wire grid floors and acclimatized for 30 min. This method consisted of evoking a hind paw flexion reflex with a hand-held force transducer (electronic analgesimeter; model EFF 301, Insight®, Ribeirão Preto, SP, Brazil) adapted with a polypropylene tip. Stimulus intensity was obtained by averaging three measurements taken with minimal intervals of 180s. The mechanical threshold measurements were performed three times for each animal and results were analyzed considering the mean value, at the following phases: before the first injection of acid saline; 24 h after the second injection of acid saline; and 1h after treatments application of each therapeutic resource on consecutive days of treatment.

2.10. Grip strength test

The hindpaw and forepaw grip force was tested as previously described Quintans-Júnior et al. [13]. The mice were allowed acclimate in apparatus twice by day for 2 days by performing the grip meter strength (Insight®, Ribeirão Preto-SP, Brazil). The evaluation consisted in holding the mice on a metal grid, so that it tended to move to the opposite side, and to observe the force exerted, in grams (g), by the animal's paws in the attempt to escape. The measurements were performed to obtain the arithmetic mean of three values on consecutive days of treatment.

2.11. Open field (OF)

The apparatus was a circular arena with base made of wood (diameter = 84cm, height = 35cm) without the ceiling. The animals were placed in the center of the apparatus for free exploration during 5 min. The session was recorded by a camera placed above the OF at height of 230 cm and the behavioral parameters were registered by an animal tracking software (Any-maze, Stoelting, USA). The behavioral evaluation included distance total traveled (in meters) and the average speed (in meters/second). This evaluation occurred on day 6 of the experiment.

2.12. Novel Object Recognition Test (NOR)

The task was carried out in the same arena used in the OF test. Throughout the repetitions of the sessions, different objects were used, each with two copies, being of the same material differing in color, size and shape. The objects were weighed enough not to be moved by animals. After each series the objects used as well as the open field were cleaned with 5% alcohol to avoid the presence of olfactory tips. In training session, animals were exposed to two identical objects for 5 min. The same procedure was carried 1 h later (test session, duration 5 min), except that one of the objects was replaced for a new one. The sessions were registered by Any-maze. The time spent exploration each object was measured in the sessions and included touching with forepaws or nose, sniffing and biting the objects. The percent time exploring each object (time exploring old or new object/time exploring both object) was calculated. This evaluation occurred on day 7 of the experiment.

2.13. Elevated plus-maze test (EPM)

The test was carried out in an apparatus made of wood containing to two open arms (27.5 x 6.5 cm) and two enclosed (27.5 x 6.5 x 18 cm). After 60 min of the treatments animals were placed individually in the center of the apparatus for free exploration during 5 min. The session was registered by Any-maze. The behavioral evaluation included the time spent exploration of the open and enclosed arms. Moreover, distance total traveled (in meters) and the average speed (in meters/second) was calculated for each animal, registering the number of entries in the open/ enclosed arms and the respective residence time. The evaluation occurred on days 8 and 9 of the experiment.

2.10. Oxidative damage markers

Proteins oxidative damage and effects on lipids in mice were analyzed in tissue samples of brain. The oxidative status of the thiol groups was assessed by quantification of the total reduced sulfhydryl (SH) groups. Samples were reacted with 5,50-dithionitrobis 2-nitrobenzoic acid (10 mM) during a 60min incubation at room temperature, and the absorbance of the solution was read using a spectrophotometer at 412 nm [35].

Lipoperoxidation was determined by the quantification of TBARS generated from the reaction of the thiobarbituric acid (TBA) with lipoperoxides in an acid-heating medium. After precipitation with 15% trichloroacetic acid, the supernatant was mixed with 0.67% TBA and heated in a boiling water bath for 20 min. TBARS was determined by measuring the absorbance at 532 nm [36].

Superoxide dismutase assay (SOD) was determined from the inhibition of superoxide anion-dependent epinephrine autoxidation [37]. Different concentrations of protein were incubated at 32˚C with adrenaline and glycine buffer (pH 10.2) for 30 min and the absorbance at 480 nm was determined from zero to 30 min, in intervals of 30 s. And the results were expressed as units of SOD/mg protein [38].

Catalase assay (CAT) was assayed by measuring the ratio of decrease in hydrogen peroxide (H_2O_2) [39]. Thus, 50µg of protein was incubated at 32˚C with H_2O_2 for 10 min and the absorbance at 240 nm was determinate at intervals of 15 s for 5 min.

2.11. Statistical analysis

Statistical differences between the treated and control groups were evaluated by One way-ANOVA or two way-ANOVA followed by Bonferroni's post hoc test, Tukey's post hoc test or t-Student-Newman-Keuls test. Differences were considered statistically significant when $p < 0.05$. And all statistical analyses were performed using Graph Pad Prism (version 5.0) (Graph Pad Prism Software Inc., San Diego, CA, USA). Results were expressed as mean and standard error of the mean (mean ± SEM).

3. Results

Figure 1 (A) represents the DSC curves of MYR, βCD, PM, KN and SC. MYR presented two events in the temperature range of 30-280 °C, corresponding to volatilization, followed by the decomposition. The curve of βCD showed a broad thermal event between 30 and 113 °C corresponding to water release, a second endothermic event in the range of 209-234 °C, characteristic of the crystalline phase transition and at 282-327 °C a thermal event attributed to the melting peak, followed by degradation of the βCD. The DSC curves of PM presented

four events like βCD, the KN and SC were observed disappearance of an endothermic peak between 209-234 °C suggesting interaction between MYR and βCD.

<INSERT FIGURE 1>

The TG/DTG analysis curves illustrated (Figure 1 B) the mass loss percentages, calculated from specific temperature ranges, for the MYR and the respective inclusion complexes, were shown in Table 1. On the curves TG/DTG, observed that the MYR lost 88.23% (30-170 ° C) and the βCD had a loss of 13.68% of its total mass. The PM, KN and SC lost 22.93%, 11.98% and 14.25%, respectively. In the second stage (170-280 °C), we observed that PM, KN and SC presented a mass loss of 1.28%, 4.55% and 3.88%, respectively. In the third phase, KN and SC showed a greater loss of mass, possibly due to the release of MYR present in the internal cavity of βCD. There results suggested possible inclusion complexes formation by KN and SC methods.

<INSERT TABLE 1>

Figure 2 displays the photomicrographs obtained with Scanning Electron Microscopy (SEM) and represents the morphological alterations linked to the host-guest interactions. The results show that βCD, PM and KN present square and rectangular crystals of different sizes. SEM of SC exhibited loss of rectangular-shaped crystals and smaller particles, suggesting interaction between MYR and βCD by this method.

<INSERT FIGURE 2>

The diffractograms of βCD, MF, KN and SC were shown in Figure 3. It was possible to observe that the βCD peaks presented characteristic pattern of a crystalline compound, as well as in KN and SC. However, in the diffractograms of KN and SC the disappearance and the appearance of new peaks were also observed, suggesting the formation of a new crystalline phase due to the interaction MYR/βCD.

<INSERT FIGURE 3>

The results of the hyperalgesia evaluation showed that 24 h after the second injection of acid saline in the gastrocnemius muscle there was a decrease in paw withdrawal threshold and this effect persisted throughout all the evaluation days. MYR/βCD group showed an increase of the paw withdrawal threshold for a period about 6 h when compared to control ($p < 0.001$). However, no effects were observed in the MYR group ($p > 0.05$, Figure 4A).

In the daily assessment, MYR/βCD group demonstrated a significant reduction of hyperalgesic behavior when compared to control group ($p < 0.001$). MYR alone exhibit significant changes in painful behavior only on the days 9 and 14. In addition, no tolerance effects were observed in the MYR and MRY/ βCD groups (Figure 4B), as well as muscle strength (Figure 4C).

<INSERT FIGURE 4>

No differences were observed distance traveled and speed mean in the treated-groups (Figure 5A and 5B).

<INSERT FIGURE 5>

In NOR test no differences was observed among groups in the training session (Figure 6A). In the test session differences were found for the saline acid animals, suggesting deficit in short-term memory. On the other hand, PGB and MYR/βCD groups spent more time exploring the new object in comparison to the familiar object (Figure 6B).

<INSERT FIGURE 6>

In relation to EPM test, PGB and MYR/ MYR/βCD groups showed an increase in the percentage of time spent in the open arms when compared to control exploration time ($p < 0.05$, Figure 7C), suggesting decrease in anxiety-like behavior. No differences were found in mean speed and distance traveled (Figure 7 A and B).

<INSERT FIGURE 7>

As showed in Figure 8, some oxidative parameters were assessed. Results demonstrated that MYR/βCD significantly decreased ($p<0.05$) MDA formation, consistent with

lipid peroxidation, in brain of FM mice (Figure 8A). Moreover, Figure 8D and E demonstrated a significant upregulated catalase activity ($p<0.05$) and a significant ($p<0.05$) decreased SOD/CAT ratio to MYR/βCD group when compared with controls. No alterations were found in SH and SOD (Figure 8B and 8C).

<INSERT FIGURE 8>

4. Discussion

FM is considered one of the most important non-inflammatory rheumatic diseases due to the related comorbidities and the difficulty of treatment, as it is a type of 'dysfunctional pain' has been considered as a 'neglected pain' due poor therapeutic options today [11, 15, 40]. Thus, the search for new therapeutic options is a great challenge for modern medicine and PNs have been shaping an inexhaustible source of new chemical entities.

Herewith we investigated the pharmacological effects of the inclusion complex containing MYR and βCD in mice submitted to a chronic widespread non-inflammatory muscle pain model (an animal model for FM). Our main results showed anti-hyperalgesic, anxiolytic like behavior and antioxidant profile.

Different analytical techniques, such as DSC, TG/DTG, SEM and XRD, were used to characterize and compare the physicochemical properties of MYR and βCD prepared solids, investigating and comparing the potential and efficacy of different preparation methods. The DSC curves of PM presented four events like βCD, the KN and SC were observed disappearance of an endothermic peak between 209-234 °C suggesting interaction between MYR and βCD. Previous studies reported similar results to others NPs such as were observed in the carvacrol/βCD [40] and limonene/βCD complexes [28].

In TG analysis, the PM, KN and SC lost in different stages also were observed in other studies with inclusion complex carvacrol/β-CD [32]. According to Hadaruga et al. [41] the first weight loss corresponding to water release, takes place in the complexation process because some water molecules are replaced by hydrophobic molecules. The paste and slurry complexes, weight loss continued after 250ºC, indicating that the inclusion complex increases the stability of monoterpenes [42].

By data analysis obtained by SEM is possible to investigate of the morphological aspects of the solid surface, helping to evidence morphological alterations that may be related to host-host interactions, besides providing information about possible new solid phases [43].

In one of the studies carried by our group, Quintans et al. [33] reported similar results in the hecogenin acetate/βCD complexes according that the new solid phase formation was characteristic of inclusion complexes between curcumin and βCD [44]. The complexation between MYR and βCD by the KN and SC methods were represented as agglomerates. In contrast, the particle morphology corresponding to MF was like the β-CD. These differences are due to the stacking interactions that occur with the CDs compared to the complexes formed but the morphology of the complex is not the only indicator of complex formation [45].

Considering the data obtained of the characterization, we demonstrated the formation of the complex containing MYR and βCD by the KN and SC methods. These results suggest that is feasible to study the pharmacological action of MYR/βCD obtained by SC method.

Afterward we confirmed the formation of the inclusion complex containing MYR/βCD and starting from the background of the analgesic profile already described for this terpene, we assessed it in a mice fibromyalgia-like model. We chose the model described by Sluka et al. [34] due the consistent hyperalgesia provoked by saline acid injection administered within gastrocnemius muscle, producing a chronic widespread non-inflammatory muscle pain and due other behavioral changes that are also related to the symptoms presented by fibromyalgic patients [34, 46]. Therefore, MYR/βCD had significant anti-hyperalgesic profile while the MYR alone exhibit significant changes in painful behavior in this animal model only on the days 9 and 14 maybe by pharmacokinetic parameters. Thus, the complexation with βCD was essential to provide an improvement of pharmacological effect of MYR when administered by oral route.

These noncovalent complexes offer a variety of physicochemical advantages drugs including the possibility for increased water solubility and solution stability [47]. Recent studies have reported that βCD can improve the anti-hyperalgesic effects of terpenes, mainly monoterpene, which appears to be related to the improvement of bioavailability and physico-chemical properties [14, 25, 28, 33]. Recent systematic review corroborates with our hypothesis which associates the use of CDs to improve the efficacy and bioavailability of analgesic and anti-inflammatory compounds already available from the pharmaceutical market, especially when the active principle is an apolar compound [21, 24, 48, 49]. Interestingly, this approach has suggested that it can be a way to reduce the therapeutic doses improving pharmacological efficacy and the possibility of adverse events related to the use of drugs as well as their potential toxicity [48, 49]. To the best of our knowledge, this is the first scientific report showing a

possible applicability of MYR under chronic conditions, such as FM to reduce hyperalgesia and anxiety.

MYR/βCD was also able to increase mechanical hyperalgesia withdrawal thresholds in chronic assay with no muscle relaxation. Although the antinociceptive and anti-inflammatory properties of MYR has already been described for other experimental models (acute models) little is known about its CNS effects or myorelaxant [16, 18]. MYR can act in the glutamatergic system [18] and high concentrations of glutamate and receptor NMDA in posterior insula have play important role in the FM pathophysiology [49, 50]. Moreover, glutamate and its receptors are important targets for anti-hiperalgesyc drugs because the activation of glutamatergic system is associated to the development and maintenance of hyperalgesia [51, 52].

Furthermore, MYR can acts as positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors, thereby it may augmenting phasic and tonic GABAergic inhibition [17, 51]. This modulation contributes to depressant effect of CNS but not affect muscle capacity, a side-effect common in the drugs used in FM treatment management [51].

The CNS depression and muscle relaxation effect can reduce the response of motor coordination and might upset the painful behavior test results found in FM animal model [13, 52]. So, we analyzed the muscle relaxation of MYR/βCD on the grip-strength meter, so unchanged force was observed in treated animals. The results reinforce the hypothesis that MYR produces anti-hyperalgesic profile, but it was not due to a feasible muscle relaxation or inhibitory effect on the CNS, at least in tested doses. It was corroborated by Silva et al. [18] that equally reported that the antinociceptive effect exerted by MYR is not related to changes in the motor coordination of animals.

The possible effect on the emotionality in any experimental FM animal models is rare in the literature, which is very strange and unaccountable because FM comorbidities are an abyss to overcome in the clinic approaches [52]. Herein we also assessed how the treatment with MYR or MYR/βCD may influence in the behavior of animals.

The Open-field test (OF) are usually explained in terms of emotionality, stress-susceptibility, exploration or coping style [53]. We performed the OF to evaluate locomotor and exploratory activities (considered also an important aspect in the emotionality), so our results demonstrated that pretreatment with MYR or MYR/βCD do not alter the locomotor activity of the animals. Moreira et al. [16] reported that MYR-treated (25, 50 and 75 mg/kg i.p) mice did not alterations on the number of crossing, grooming or rearing when compared with a negative control in OF. The unchanged motor coordination tests can be important because other animal models which evaluate cognitive or potential anxiolytic-like behavior, such as elevated

plus-maze test (EPM) and new object recognition task (NOR), can present false positive results if the motor function have altered [16].

NOR was used to investigate associative memory, since this task quantify exploration of objects, and delimiting memory settings [54]. Our results indicated short-term memory deficit in FM animals, but not in the MYR/βCD and PGB-treated mice, so showing a promising profile. Pain and depressive symptoms in FM patients are related, at least in part, to significant deficits in emotionally charged cognitive tasks and mainly affective processes involved in learning, memory, attention, and decision-making [55]. Thus, our result can contribute to study of reduction this comorbidity for FM patients.

Furthermore, MYR/βCD and pregabalin (PGB), one of the three most important drugs used in the FM symptoms management, increased exploration time on open arm, so suggesting a possible upgrade emotionality of mice reducing an anxiety-like behavior. This result is newsworthy because the behaviors as pain catastrophizing, fear of pain, and pain severity are associated with anxiety in patients with FM [56]. The anxiolytic-like effect of this monoterpene can be mediated by GABAergic transmission because MYR appears to improve GABA neurotransmission via $GABA_A$ activation [16]. It was also demonstrated that other terpenes such as carvacrol [57], linalool [58], 1,4-cineole [59] and phytol [60] presented a similar effects of the MYR that seem to be driven by the ease of crossing the blood-cerebrospinal barrier [61]. In fact, the monoterpenes are molecules formed by two isoprene subunits that when showed any CNS depressor profile, it seems to be associated with modulation of GABAergic system [62, 63]. In this context, MYR showed a similar profile to PGB which has been used to generalized anxiety disorder, social anxiety disorder and bipolar disorder [64], because inhibit calcium influx and subsequent release of excitatory neurotransmitters [65].

FM provokes a disrupt in redox homeostasis, causing an oxidative damage and decreasing antioxidant defenses [9]. Moreover, several studies have shown mitochondrial dysfunction and high levels of oxidative stress markers in FM patients [66, 67]. The inseparable relationship between the increase of ROS and the reduction of endogenous antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT) and glutathione, seems to be already documented with the symptoms of FM [68].

However, little is known about oxidative stress in FM-positive animals. The oxidative state of the cerebrospinal fluid (CSF) in a mice FM-like model was recently evaluated by Klein et al. [69]. Although the authors used an different FM animal model which experimental syndrome was induced by reserpine, the pretreatment with PGB, resveratrol and Rice Oil reduced reactive oxygen species (ROS) in CSF and suppressed behaviors related to FM

symptoms [69]. Brain oxidative stress has been implicated in the response to stress and in the pathogenesis of neurologic and psychiatric diseases, as well as to FM syndrome [70].

To better understand if the balance of oxidative stress may be related to the pharmacological effect produced by MYR in mice FM-like model, we assessed the redox equilibrium in brain from mice of control and MYR/βCD groups. So, we demonstrated that MYR/βCD can prevent oxidative imbalance by decrease oxidative damage in lipids and by upregulate catalase activity which produces a protective profile of oxidative stress in the CNS. The increased lipid peroxidation can disrupt the function of biological membranes [71, 72] and is often associated with cognitive deficit [73]. Further, the catalase is an enzyme that metabolizes H_2O_2, releasing water and oxygen, ergo H_2O_2 is one of the main indicator of brain homeostasis and disturbance in H_2O_2 contend in cerebral structures was related to brain diseases [74]. Thus, the maintenance of oxidative homeostasis induces by MYR/βCD could be potentially underlying in the improvement of behavioral impairment observed in treated animals.

Moreover, our result is corroborated, at least in part, by the fact that the MYR can restore the impairment of endogenous antioxidant enzymes activities [19]. Oral doses of 25, 50 and 100 mg/kg was able to significantly decrease the activity of myeloperoxidase and malondialdehyde, as also can increase in glutathione peroxidase, SOD, and catalase activity in gastric tissues [17].

The protective role of alcoholic terpenes, as MYR, in neuroprotection in animal models that induce strong oxidative stress seems to be easily explained by the presence of its hydroxyl group [75]. Thus, MYR is a probably donates hydrogen atoms with an unpaired electron (H•), producing another radical that is stabilized by electron scattering generated at a molecule resonance structure, similarly to what occurs with terpenes such as carvacrol [75] and linalool [76]. This protective effect of redox balance in rodents in this specific mice FM-like model produced by MYR is new and needs to be better explored in future work.

5. Conclusion

Together, the present study suggests that MYR can be complexed in βCD which is able to produce a very significant reduction of mechanical hyperalgesia, to produces an anxiolytic-like profile, preventing cognitive alterations caused by an animal model for FM. These findings seem to be related to maintenance of redox homeostasis induced by MYR/βCD. The complexation with βCD could enhances the pharmacological profile of this terpene maybe by the increase of bioavailability or by a greater water solubility of the active compound that

favors its biological effect. However, more studies are required for elucidate the MYR central action mechanisms. Thus, the results found here are stimulants for the continuation of studies with MYR or MYR/βCD in this painful syndrome seeking new therapeutic proposals for FM patients which can supply the pain and ameliorate other comorbidities equally deleterious for them.

Conflict of interest

All authors report no conflict of interest.

Acknowledgements

This work was supported by grants from CNPq, FAPITEC/SE, CAPES and FINEP, all from Brazil.

References

[1] F. Wolfe, D.J. Clauw, M.A. Fitzcharles, D.L. Goldenberg, R.S. Katz, P. Mease, A.S. Russell, I.J. Russell, J.B. Winfield, M.B. Yunus, The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity, Arthritis Care Res. 62 (2010) 600–610. doi:10.1002/acr.20140.

[2] B.T.E. Dymon, D. Pharm, S.J. Rogers, D. Pharm, D.M. Hunsinger-norris, D. Pharm, B. Aq-cardiology, Fibromyalgia, Acsap. (2015) 5–18.

[3] A. Cabo-Meseguer, G. Cerdá-Olmedo, J.L. Trillo-Mata, Fibromyalgia: Prevalence, epidemiologic profiles and economic costs, Med. Clínica (English Ed. (2017). doi:10.1016/j.medcle.2017.10.011.

[4] F. Heidari, M. Afshari, M. Moosazadeh, Prevalence of fibromyalgia in general population and patients, a systematic review and meta-analysis, Rheumatol. Int. 37 (2017) 1527–1539. doi:10.1007/s00296-017-3725-2.

[5] J.E. Sumpton, D.E. Moulin, Fibromyalgia, Handb. Clin. Neurol. 119 (2014) 513–527. doi:10.1016/B978-0-7020-4086-3.00033-3.

[6] D.J. Wallace, I.M. Gavin, O. Karpenko, F. Barkhordar, B.S. Gillis, Cytokine and chemokine profiles in fibromyalgia, rheumatoid arthritis and systemic lupus erythematosus: A potentially useful tool in differential diagnosis, Rheumatol. Int. 35 (2015) 991–996. doi:10.1007/s00296-014-3172-2.

[7] A. Ranzolin, A.L.B.P. Duarte, M. Bredemeier, C.A. da Costa Neto, B.M. Ascoli, B.

Wollenhaupt-Aguiar, F. Kapczinski, R.M. Xavier, Evaluation of cytokines, oxidative stress markers and brain-derived neurotrophic factor in patients with fibromyalgia - A controlled cross-sectional study, Cytokine. 84 (2016) 25–28. doi:10.1016/j.cyto.2016.05.011.

[8] C.P. Chung, D. Titova, A. Oeser, M. Randels, I. Avalos, G.L. Milne, J.D. Morrow, C.M. Stein, Oxidative stress in fibromyalgia and its relationship to symptoms, Clin. Rheumatol. 28 (2009) 435–438. doi:10.1007/s10067-008-1072-0.

[9] G. Fatima, S.K. Das, A.A. Mahdi, Some oxidative and antioxidative parameters and their relationship with clinical symptoms in women with fibromyalgia syndrome, Rev. Bras. Reumatol. 47 (2017) 354–361. doi:10.1111/1756-185X.12550.

[10] R.C. Dutra, M.M. Campos, A.R.S. Santos, J.B. Calixto, Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives, Pharmacol. Res. 112 (2016) 4–29. doi:10.1016/j.phrs.2016.01.021.

[11] Y. Nagakura, Challenges in drug discovery for overcoming “dysfunctional pain”: an emerging category of chronic pain., Expert Opin. Drug Discov. 10 (2015) 1043–5. doi:10.1517/17460441.2015.1066776.

[12] S.S. Nascimento, E.A. Camargo, J.M. Desantana, A.A.S. Araújo, P.P. Menezes, W. Lucca-Júnior, R.L.C. Albuquerque-Júnior, L.R. Bonjardim, L.J. Quintans-Júnior, Linalool and linalool complexed in β-cyclodextrin produce anti-hyperalgesic activity and increase Fos protein expression in animal model for fibromyalgia, Naunyn. Schmiedebergs. Arch. Pharmacol. 387 (2014) 935–942. doi:10.1007/s00210-014-1007-z.

[13] L.J. Quintans-Júnior, A.A.S. Araújo, R.G. Brito, P.L. Santos, J.S.S. Quintans, P.P. Menezes, M.R. Serafini, G.F. Silva, F.M.S. Carvalho, N.K. Brogden, K.A. Sluka, β-caryophyllene, a dietary cannabinoid, complexed with β-cyclodextrin produced anti-hyperalgesic effect involving the inhibition of Fos expression in superficial dorsal horn, Life Sci. 149 (2016) 34–41. doi:10.1016/j.lfs.2016.02.049.

[14] P.S. Siqueira-Lima, R.G. Brito, H.G. Araújo-Filho, P.L. Santos, A. Lucchesi, A.A.S. Araújo, P.P. Menezes, L. Scotti, M.T. Scotti, I.R.A. Menezes, H.D.M. Coutinho, G. Zengin, A. Aktumsek, A.R. Antoniolli, L.J. Quintans-Júnior, J.S.S. Quintans, Anti-hyperalgesic effect of Lippia grata leaf essential oil complexed with β-cyclodextrin in a chronic musculoskeletal pain animal model: Complemented with a molecular docking and antioxidant screening, Biomed. Pharmacother. 91 (2017) 739–747. doi:10.1016/j.biopha.2017.05.009.

[15] M.A. Oliveira, A.G. Guimarães, A.A.S. Araújo, L.J. Quintans-Júnior, J.S.S. Quintans, New drugs or alternative therapy to blurring the symptoms of fibromyalgia—a patent review, Expert Opin. Ther. Pat. 27 (2017) 1147–1157. doi:10.1080/13543776.2017.1349105.

[16] M.R.C. Moreira, M.G. da S.S. Salvadori, A.A.C. de Almeida, D.P. de Sousa, J. Jordán, P. Satyal, R.M. de Freitas, R.N. de Almeida, Anxiolytic-like effects and mechanism of (-)-myrtenol: A monoterpene alcohol, Neurosci. Lett. 579 (2014) 119–124. doi:10.1016/j.neulet.2014.07.007.

[17] A.F.S.C. Viana, F.V. da Silva, H. de B. Fernandes, I.S. Oliveira, M.A. Braga, P.I.G. Nunes, D. de A. Viana, D.P. de Sousa, V.S. Rao, R. de C.M. Oliveira, F. Almeida Santos, Gastroprotective effect of (-)-myrtenol against ethanol-induced acute gastric lesions: possible mechanisms, J. Pharm. Pharmacol. (2016) 1085–1092. doi:10.1111/jphp.12583.

[18] R.O. Silva, M.S. Salvadori, F.B.M. Sousa, M.S. Santos, N.S. Carvalho, D.P. Sousa, B.S. Gomes, F.A. Oliveira, A.L.R. Barbosa, R.M. Freitas, R.N. de Almeida, J.V.R. Medeiros, Evaluation of the anti-inflammatory and antinociceptive effects of myrtenol, a plant-derived monoterpene alcohol, in mice, Flavour Fragr. J. 29 (2014) 184–192. doi:10.1002/ffj.3195.

[19] R.M. de Britto, J.A. da S. Neto, T.R.R. Mesquita, C.M.L. de Vasconcelos, G.K.M. de Almeida, I.C.G. de Jesus, P.H. dos Santos, D.S. de Souza, R. Miguel-dos-Santos, L.A. de Sá, F.S.M. dos Santos, R.N. Pereira-Filho, R.L.C. Albuquerque-Júnior, L.J. Quintans-Júnior, S. Guatimosim, S. Lauton-Santos, Myrtenol protects against myocardial ischemia-reperfusion injury through antioxidant and anti-apoptotic dependent mechanisms, Food Chem. Toxicol. (2017). doi:10.1016/j.fct.2017.12.003.

[20] J. De Souza Siqueira Quintans, P.P. Menezes, M.R.V. Santos, L.R. Bonjardim, J.R.G.S. Almeida, D.P. Gelain, A.A.D.S. Araújo, L.J. Quintans, Improvement of p-cymene antinociceptive and anti-inflammatory effects by inclusion in β-cyclodextrin, Phytomedicine. 20 (2013) 436–440. doi:10.1016/j.phymed.2012.12.009.

[21] P.S.S. Lima, A.M. Lucchese, H.G. Araújo-Filho, P.P. Menezes, A.A.S. Araújo, L.J. Quintans-Júnior, J.S.S. Quintans, Inclusion of terpenes in cyclodextrins: Preparation, characterization and pharmacological approaches, Carbohydr. Polym. 151 (2016) 965–987. doi:10.1016/j.carbpol.2016.06.040.

[22] R.N. Marreto, E.E.C.V. Almeida, P.B. Alves, E.S. Niculau, R.S. Nunes, C.R.S. Matos, A.A.S. Araújo, Thermal analysis and gas chromatography coupled mass spectrometry analyses of hydroxypropyl-β-cyclodextrin inclusion complex containing Lippia gracilis

essential oil, Thermochim. Acta. 475 (2008) 53–58. doi:10.1016/j.tca.2008.06.015.

[23] P.J. Salústio, P. Pontes, C. Conduto, I. Sanches, C. Carvalho, J. Arrais, H.M.C. Marques, Advanced Technologies for Oral Controlled Release: Cyclodextrins for Oral Controlled Release, AAPS PharmSciTech. 12 (2011) 1276–1292. doi:10.1208/s12249-011-9690-2.

[24] G. de Oliveira Makson, A.G. Guimarães, A. Araújo Adriano, S. Quintans Jullyana, M.R. Santos, L.J. Quintans-Júnior, Cyclodextrins: improving the therapeutic response of analgesic drugs: a patent review, Expert Opin. Ther. Pat. 25 (2015) 897–907. doi:10.1517/13543776.2015.1045412.

[25] P.L. Santos, R.G. Brito, M.A. Oliveira, J.S.S. Quintans, A.G. Guimarães, M.R.V. Santos, P.P. Menezes, M.R. Serafini, I.R.A. Menezes, H.D.M. Coutinho, A.A.S. Araújo, L.J. Quintans-Júnior, Docking, characterization and investigation of β-cyclodextrin complexed with citronellal, a monoterpene present in the essential oil of Cymbopogon species, as an anti-hyperalgesic agent in chronic muscle pain model, Phytomedicine. 23 (2016) 948–957. doi:10.1016/j.phymed.2016.06.007.

[26] M.R. Serafini, P.P. Menezes, L.P. Costa, C.M. Lima, L.J. Quintans, J.C. Cardoso, J.R. Matos, J.L. Soares-Sobrinho, S. Grangeiro, P.S. Nunes, L.R. Bonjadim, A.A.S. Araújo, Interaction of p-cymene with b-cyclodextrin, in: J. Therm. Anal. Calorim., 2012: pp. 951–955. doi:10.1007/s10973-011-1736-x.

[27] M. Ceborska, K. Szwed, M. Asztemborska, M. Wszelaka-Rylikb, E. Kicinska, K. Suwinska, Study of b-cyclodextrin inclusion complexes with volatile molecules geraniol and g-terpineol enantiomers in solid state and in solution, Chem. Phys. Lett. 641 (2015) 44–50. doi:10.1016/j.cplett.2015.10.018.

[28] H.G. Araújo-Filho, E.W.M. Pereira, M.M. Rezende, P.P. Menezes, A.A.S. Araújo, R.S.S. Barreto, A.O.B.P.B. Martins, T.R. Albuquerque, B.A.F. Silva, I.S. Alcantara, H.D.M. Coutinho, I.R.A. Menezes, L.J. Quintans-Júnior, J.S.S. Quintans, D-limonene exhibits superior antihyperalgesic effects in a β-cyclodextrin-complexed form in chronic musculoskeletal pain reducing Fos protein expression on spinal cord in mice, Neuroscience. 358 (2017) 158–169. doi:10.1016/j.neuroscience.2017.06.037.

[29] T.A. Andrade, T.S. Freitas, F.O. Araújo, P.P. Menezes, G.A.A. Dória, A.S. Rabelo, L.J. Quintans-Júnior, M.R.V. Santos, D.P. Bezerra, M.R. Serafini, I.R.A. Menezes, P.S. Nunes, A.A.S. Araújo, M.S. Costa, F.F. Campina, A.T.L. Santos, A.R.P. Silva, H.D.M. Coutinho, Physico-chemical characterization and antibacterial activity of inclusion complexes of Hyptis martiusii Benth essential oil in β-cyclodextrin, Biomed. Pharmacother. 89 (2017) 201–207. doi:10.1016/j.biopha.2017.01.158.

[30] K. Uekama, F. Hirayama, T. Irie, Cyclodextrin drug carrier systems, Chem. Rev. 98 (1998) 2045–2076. doi:10.1021/cr970025p.

[31] E. Pinho, M. Grootveld, G. Soares, M. Henriques, Cyclodextrins as encapsulation agents for plant bioactive compounds, Carbohydr. Polym. 101 (2014) 121–135. doi:10.1016/j.carbpol.2013.08.078.

[32] P. dos P. Menezes, M.R. Serafini, Y.M.B.G. de Carvalho, D.V. Soares Santana, B.S. Lima, L.J. Quintans-Júnior, R.N. Marreto, T.M. de Aquino, A.R. Sabino, L. Scotti, M.T. Scotti, S. Grangeiro-Júnior, A.A. de Souza Araújo, Kinetic and physical-chemical study of the inclusion complex of β-cyclodextrin containing carvacrol, J. Mol. Struct. 1125 (2016) 323–330. doi:10.1016/j.molstruc.2016.06.062.

[33] J.S.S. Quintans, E.W.M. Pereira, Y.M.B.G. Carvalho, P.P. Menezes, M.R. Serafini, M.V.A. Batista, C.D.L.F.A. Moreira, Á.A.N. Lima, A. Branco, J.R.G.S. Almeida, D.P. Gelain, G. Zengin, A.A.S. Araújo, L.J. Quintans-Júnior, Host–guest inclusion complexation of β-cyclodextrin and hecogenin acetate to enhance anti-hyperalgesic effect in an animal model of musculoskeletal pain, Process Biochem. 59 (2017) 123–131. doi:10.1016/j.procbio.2016.08.025.

[34] K.A. Sluka, A. Kalra, S.A. Moore, Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia, Muscle and Nerve. 24 (2001) 37–46. doi:10.1002/1097-4598(200101)24:1<37::AID-MUS4>3.0.CO;2-8.

[35] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77. doi:10.1016/0003-9861(59)90090-6.

[36] H.H. Draper, M. Hadley, Malondialdehyde determination as index of lipid Peroxidation, Methods Enzymol. 186 (1990) 421–431. doi:10.1016/0076-6879(90)86135-I.

[37] H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase., J. Biol. Chem. 247 (1972) 3170–3175. doi:4623845.

[38] L. Heimfarth, J. Delgado, M.R. Mingori, K.S. Moresco, R.P. Pureur, D.P. Gelain, J.C.F. Moreira, Delayed neurochemical effects of prenatal exposure to MeHg in the cerebellum of developing rats, Toxicol. Lett. 284 (2018) 161–169. doi:10.1016/j.toxlet.2017.12.006.

[39] H. Aebi, [13] Catalase in Vitro, Methods Enzymol. 105 (1984) 121–126. doi:10.1016/S0076-6879(84)05016-3.

[40] A.G. Guimarães, M.A. Oliveira, R.D.S. Alves, P.D.P. Menezes, M.R. Serafini, A.A. De Souza Araújo, D.P. Bezerra, L.J. Quintans, Encapsulation of carvacrol, a monoterpene present in the essential oil of oregano, with β-cyclodextrin, improves the

pharmacological response on cancer pain experimental protocols, Chem. Biol. Interact. 227 (2015) 69–76. doi:10.1016/j.cbi.2014.12.020.

[41] N.G. Hădărugă, D.I. Hădărugă, H.D. Isengard, Water content of natural cyclodextrins and their essential oil complexes: A comparative study between Karl Fischer titration and thermal methods, in: Food Chem., 2012: pp. 1741–1748. doi:10.1016/j.foodchem.2011.11.003.

[42] M. Chen, J. Wang, W. Zhang, G. Diao, Preparation and characterization water-soluble inclusion complexes of imidacloprid-β-cyclodextrin polymer and their electrochemical behavior, J. Electroanal. Chem. 696 (2013) 1–8. doi:10.1016/j.jelechem.2013.02.016.

[43] M.A.M. Lyra, L.D.S. Alves, D.A.F. Fontes, J.L. Soares-Sobrinho, P.J. Rolim-Neto, Ferramentas analíticas aplicadas à caracterizaçã o de complexos de inclusão fármaco-ciclodextrina, Rev. Ciencias Farm. Basica E Apl. 31 (2010) 117–124.

[44] C.S. Mangolim, C. Moriwaki, A.C. Nogueira, F. Sato, M.L. Baesso, A.M. Neto, G. Matioli, Curcumin-β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application, Food Chem. 153 (2014) 361–370. doi:10.1016/j.foodchem.2013.12.067.

[45] S. Tenjarla, P. Puranajoti, R. Kasina, T. Mandal, Preparation Characterization Evaluation of Miconazole-Cyclodextrin Complexes for Improved Oral Topical Delivery, J. Pharm. Sci. 87 (1998) 425–429. doi:10.1021/js970361l.

[46] D. Pratt, P.N. Fuchs, K.A. Sluka, Assessment of avoidance behaviors in mouse models of muscle pain, Neuroscience. 248 (2013) 54–60. doi:10.1016/j.neuroscience.2013.05.058.

[47] T. Loftsson, M.E. Brewster, Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization, J. Pharm. Sci. 85 (1996) 1017–1025. doi:10.1021/js950534b.

[48] B. Gidwani, A. Vyas, Synthesis, characterization and application of Epichlorohydrin-β-cyclodextrin polymer, Colloids Surfaces B Biointerfaces. 114 (2014) 130–137. doi:10.1016/j.colsurfb.2013.09.035.

[49] R.E. Harris, P.C. Sundgren, A.D. Craig, E. Kirshenbaum, A. Sen, V. Napadow, D.J. Clauw, Elevated insular glutamate in fibromyalgia is associated with experimental pain, Arthritis Rheum. 60 (2009) 3146–3152. doi:10.1002/art.24849.

[50] A. Beirith, A.R.S. Santos, J.B. Calixto, Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw, Brain Res. 924 (2002)

219–228. doi:10.1016/S0006-8993(01)03240-1.

[51] E. Bellato, E. Marini, F. Castoldi, N. Barbasetti, L. Mattei, D.E. Bonasia, D. Blonna, Fibromyalgia syndrome: Etiology, pathogenesis, diagnosis, and treatment, Pain Res. Treat. 2012 (2012). doi:10.1155/2012/426130.

[52] K.A. Sluka, Is it possible to develop an animal model of fibromyalgia?, Pain. 146 (2009) 3–4. doi:10.1016/j.pain.2009.07.032.

[53] A. Ivinskis, A note on the open???field test of emotionality, Aust. J. Psychol. 18 (1966) 276–280. doi:10.1080/00049536608255550.

[54] M. Leger, A. Quiedeville, V. Bouet, B. Haelewyn, M. Boulouard, P. Schumann-Bard, T. Freret, Object recognition test in mice, Nat. Protoc. 8 (2013) 2531–2537. doi:10.1038/nprot2013155.

[55] C. Walteros, J.P. Sánchez-Navarro, M.A. Muñoz, J.M. Martínez-Selva, D. Chialvo, P. Montoya, Altered associative learning and emotional decision making in fibromyalgia, J. Psychosom. Res. 70 (2011) 294–301. doi:10.1016/j.jpsychores.2010.07.013.

[56] K. Hadlandsmyth, D.L. Dailey, B.A. Rakel, M.B. Zimmerman, C.G. Vance, E.N. Merriwether, R.L. Chimenti, K.M. Geasland, L.J. Crofford, K.A. Sluka, Somatic symptom presentations in women with fibromyalgia are differentially associated with elevated depression and anxiety, J. Health Psychol. (2017) 135910531773657. doi:10.1177/1359105317736577.

[57] F.H.C. Melo, E.T. Venâncio, D.P. De Sousa, M.M. De França Fonteles, S.M.M. De Vasconcelos, G.S.B. Viana, F.C.F. De Sousa, Anxiolytic-like effect of Carvacrol (5-isopropyl-2-methylphenol) in mice: Involvement with GABAergic transmission, Fundam. Clin. Pharmacol. 24 (2010) 437–443. doi:10.1111/j.1472-8206.2009.00788.x.

[58] V.M. Linck, A.L. da Silva, M. Figueiro, E.B. Caramao, P.R.H. Moreno, E. Elisabetsky, Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice, Phytomedicine. 17 (2010) 679–683. doi:10.1016/j.phymed.2009.10.002.

[59] P.B. Gomes, M.L. Feitosa, M.I.G. Silva, E.C. Noronha, B.A. Moura, E.T. Venâncio, E.R.V. Rios, D.P. de Sousa, S.M.M. de Vasconcelos, M.M. de F. Fonteles, F.C.F. de Sousa, Anxiolytic-like effect of the monoterpene 1,4-cineole in mice, Pharmacol. Biochem. Behav. 96 (2010) 287–293. doi:10.1016/j.pbb.2010.05.019.

[60] J.P. Costa, G.A.L. De Oliveira, A.A.C. De Almeida, M.T. Islam, D.P. De Sousa, R.M. De Freitas, Anxiolytic-like effects of phytol: Possible involvement of GABAergic transmission, Brain Res. 1547 (2014) 34–42. doi:10.1016/j.brainres.2013.12.003.

[61] Gp. Kumar, F. Khanum, Neuroprotective potential of phytochemicals, Pharmacogn.

Rev. 6 (2012) 81. doi:10.4103/0973-7847.99898.

[62] M. Kubacka, T. Librowski, R. Czarnecki, B. Frackowiak, S. Lochyński, Anticonvulsant and antidepressant activity of the selected terpene GABA derivatives in experimental tests in mice, Pharmacol. Reports. 58 (2006) 936–943.

[63] C.S. Passos, M.D. Arbo, S.M.K. Rates, G.L. Von Poser, Terpen??ides com atividade sobre o Sistema Nervoso Central (SNC), Brazilian J. Pharmacogn. 19 (2009) 140–149. doi:10.1590/S0102-695X2009000100024.

[64] D.M. Tassone, E. Boyce, J. Guyer, D. Nuzum, Pregabalin: a novel gamma-aminobutyric acid analogue in the treatment of neuropathic pain, partial-onset seizures, and anxiety disorders. [Review] [53 refs], Clin. Ther. 29 (2007) 26–48. doi:10.1016/j.clinthera.2007.01.013.

[65] H.N. Bockbrader, D. Wesche, R. Miller, S. Chapel, N. Janiczek, P. Burger, A comparison of the pharmacokinetics and pharmacodynamics of pregabalin and gabapentin, Clin. Pharmacokinet. 49 (2010) 661–669. doi:10.2165/11536200-000000000-00000.

[66] S. Ozgocmen, H. Ozyurt, S. Sogut, O. Akyol, O. Ardicoglu, H. Yildizhan, Antioxidant status, lipid peroxidation and nitric oxide in fibromyalgia: Etiologic and therapeutic concerns, Rheumatol. Int. 26 (2006) 598–603. doi:10.1007/s00296-005-0079-y.

[67] M.D. Cordero, F.J. Cano-García, E. Alcocer-Gómez, M. de Miguel, J.A. Sánchez-Alcázar, Oxidative stress correlates with headache symptoms in Fibromyalgia: Coenzyme Q 10 effect on clinical improvement, PLoS One. 7 (2012). doi:10.1371/journal.pone.0035677.

[68] M. Meeus, J. Nijs, L. Hermans, D. Goubert, P. Calders, The role of mitochondrial dysfunctions due to oxidative and nitrosative stress in the chronic pain or chronic fatigue syndromes and fibromyalgia patients: peripheral and central mechanisms as therapeutic targets?, Expert Opin. Ther. Targets. 17 (2013) 1081–1089. doi:10.1517/14728222.2013.818657.

[69] C.P. Klein, M.R. Cintra, N. Binda, D.M. Diniz, M.V. Gomez, A.A. Souto, A.H. De Souza, Coadministration of Resveratrol and Rice Oil Mitigates Nociception and Oxidative State in a Mouse Fibromyalgia-Like Model, Pain Res. Treat. 2016 (2016). doi:10.1155/2016/3191638.

[70] S. Schiavone, V. Jaquet, L. Trabace, K.-H. Krause, Severe Life Stress and Oxidative Stress in the Brain: From Animal Models to Human Pathology, Antioxid. Redox Signal. 18 (2013) 1475–1490. doi:10.1089/ars.2012.4720.

[71] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, 2007.

doi:10.1016/0891-5849(91)90055-8.

[72] S. V Avery, Molecular targets of oxidative stress, Biochem J. 434 (2011) 201–210. doi:10.1042/BJ20101695.

[73] L. Liaquat, Z. Batool, S. Sadir, S. Rafiq, S. Shahzad, T. Perveen, S. Haider, Naringenin-induced enhanced antioxidant defence system meliorates cholinergic neurotransmission and consolidates memory in male rats, Elsevier Inc, 2017. doi:10.1016/j.lfs.2017.12.034.

[74] H. Jiang, Y.C. Wu, M. Nakamura, Y. Liang, Y. Tanaka, S. Holmes, V.L. Dawson, T.M. Dawson, C.A. Ross, W.W. Smith, Parkinson's disease genetic mutations increase cell susceptibility to stress: Mutant α-synuclein enhances H2O2- and Sin-1-induced cell death, Neurobiol. Aging. 28 (2007) 1709–1717. doi:10.1016/j.neurobiolaging.2006.07.017.

[75] A.G. Guimarães, G.F. Oliveira, M.S. Melo, S.C.H. Cavalcanti, A.R. Antoniolli, L.R. Bonjardim, F.A. Silva, J.P.A. Santos, R.F. Rocha, J.C.F. Moreira, A.A.S. Araújo, D.P. Gelain, L.J. Quintans-Júnior, Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol, Basic Clin. Pharmacol. Toxicol. 107 (2010) 949–957. doi:10.1111/j.1742-7843.2010.00609.x.

[76] M.T. de Santana Souza, J.R.G. da S. Almeida, A.A. de Souza Araujo, M.C. Duarte, D.P. Gelain, J.C.F. Moreira, M.R.V. dos Santos, L.J. Quintans-Júnior, Structure-activity relationship of terpenes with anti-inflammatory profile - A systematic review, Basic Clin. Pharmacol. Toxicol. 115 (2014) 244–256. doi:10.1111/bcpt.12221.

ANEXOS

FIGURES CAPTIONS

Figure 1. DSC (A) and TG/DTG curves (B) of MYR, βCD, PM, KN and SC in the molar ratios of 1:1 in dynamic nitrogen atmosphere (100 ml.min^{-1}) and heat (10 °C.min-^{1}).

Figure 2. Scanning Electron Microscopy (SEM) images of βCD, MF, KN and SC in an increase of 300 and 700x.

Figure 3. X-ray diffractogram of βCD, MF, KN and SC.

Figure 4. Effect of acute (A) and chronic (B) administration and assessment of muscle strength (C) of MYR/βCD (50 mg/kg; p.o.), MYR (50 mg/kg; p.o.) or vehicle (saline 0,9%) on mechanical hyperalgesia induced by acidic saline in mice. Each point represents the mean ± S.E.M (n = 8, per group). ***p < 0.001 vs. control group (Two-way ANOVA followed by Bonferroni post hoc).

Figure 5. Effect of MYR/βCD (50 mg/kg; p.o.), MYR (50 mg/kg; p.o.), positive control (PGB 30mg/kg ip.) or vehicle (saline 0,9%) in the open field test in fibromialgic positive mice. Each point represents the mean ± S.E.M (n = 8, per group). * p <0.05 negative control for all other groups; #p <0.05 positive control for SHAM, MYR and MYR/βCD groups; ° p <0.05 positive control in relation to the SHAM group; ~ p <0.05 treatment effect (ANOVA followed by Tukey's post hoc).

Figure 6. Effect of MYR/βCD (50 mg/kg; p.o.), MYR (50 mg/kg; p.o.), positive control (PGB 30mg/kg ip.) or vehicle (saline 0,9%) in training session (A) and test session (B) in novel object recognition task in fibromialgic positive mice. *p <0.05 treatment effect (ANOVA followed by Tukey's post hoc).

Figure 7. Effect of MYR/βCD (50 mg/kg; p.o.), MYR (50/ mg/kg; p.o.), positive control (PGB 30mg/kg ip.) or vehicle (saline) in exploration time on open arm in EMP test in fibromialgic positive mice. *p <0.05 treatment effect when compared with negative control (ANOVA followed by Tukey's post hoc).

Figure 8. Effect of MYR/βCD (50 mg/kg; p.o.) or vehicle (saline) on parameters of oxidative imbalance in the brain of mice. A) reactive species of thiobarbituric acid (TBARS) expressed in % of control; B) total reduced thiol content (SH) expressed in µmol SH/ mg protein; C) Total SOD activity expressed in U SOD/mg protein. D) catalase activity expressed in U CAT/mg protein; E) ratio of SOD/CAT activities expressed in U SOD/U CAT. Data are reported as means ± SEM of 7 fibromialgic positive mice. Statistically significant differences from control group, as determined by T- test: *$P < 0.05$.

Table 1. Mass loss of MYR, βCD, PM, KN and SC in molar ratio 1:1

SAMPLES	Mass loss (%)			
	Δm_1	**Δm_2**	**Δm_3**	**Δm_4**
	30-170 °C	**170-280 °C**	**280-365 °C**	**365-900 °C**
MYR	88.23#	2.29	3.77	-
βCD	13.68*	0.48	62.45	23.41***
PM	22.93+	1.28+	66.84+	15.78***
KN	**11.68+**	4.55++	**74.18+++**	13.11***
SC	**14.25+**	3.88++	**71.81+++**	12.76***

Percentage of MYR 50 mg/kg; p.o.latilized up to 170 ° C; * Percentage of H_2O release up to 170 ° C; + Mass loss related to the substitution of water molecules of β-CD by the MYR up to 170ºC; +++ loss of mass probably attributed to release of MYR in the range of 260 to 370 ° C; *** formation of elemental carbon due to carbonization of the sample in the range of 370 to 900 ° C.

Figure 1.

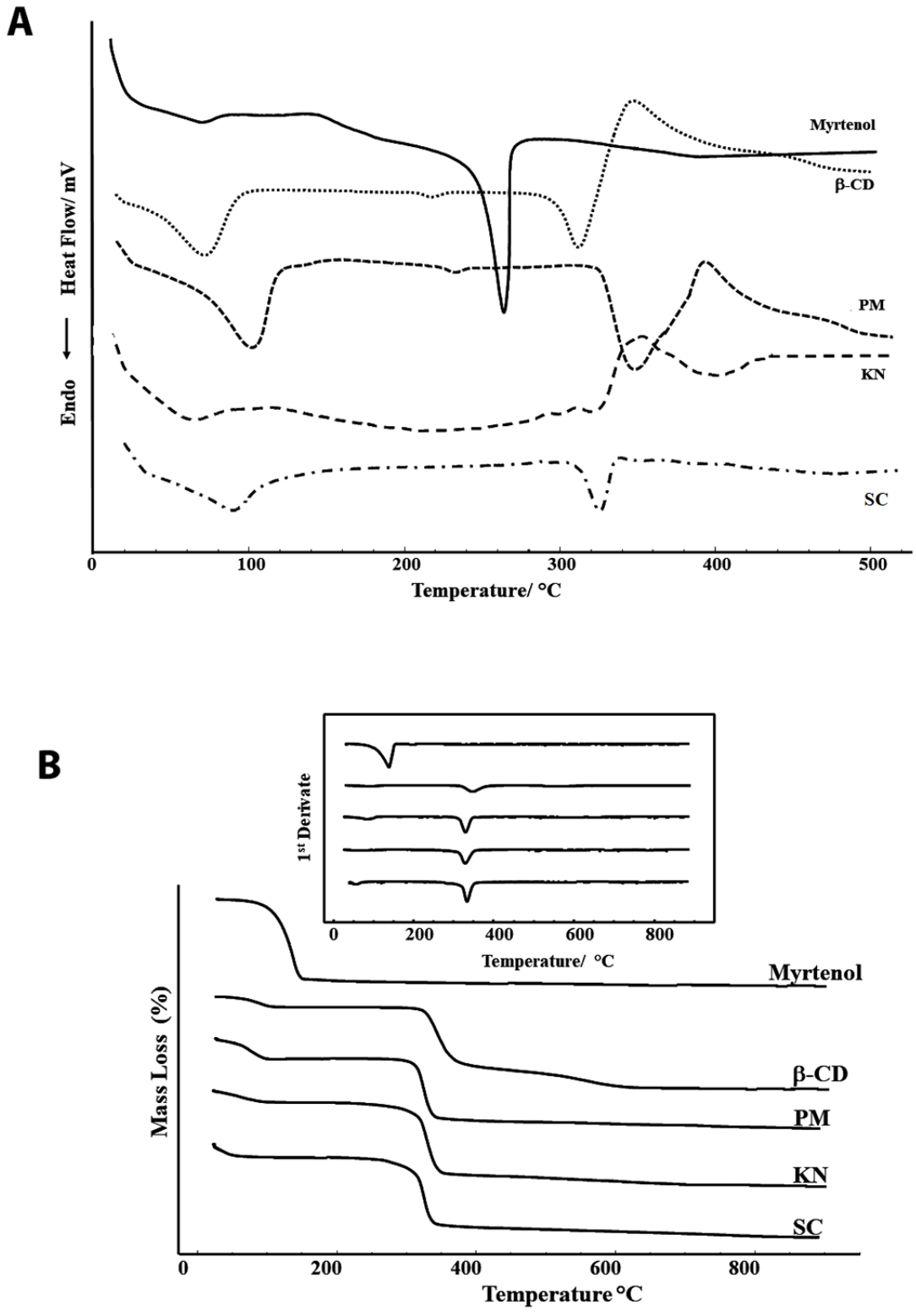

A
Endo ⟵ Heat Flow/ mV
Myrtenol
β-CD
PM
KN
SC
0
100
200
300
400
500
Temperature/ °C
B
1st Derivate
0
200
400
600
800
Temperature/ °C
Mass Loss (%)
Myrtenol
β-CD
PM
KN
SC
0
200
400
600
800
Temperature °C

Figure 2.

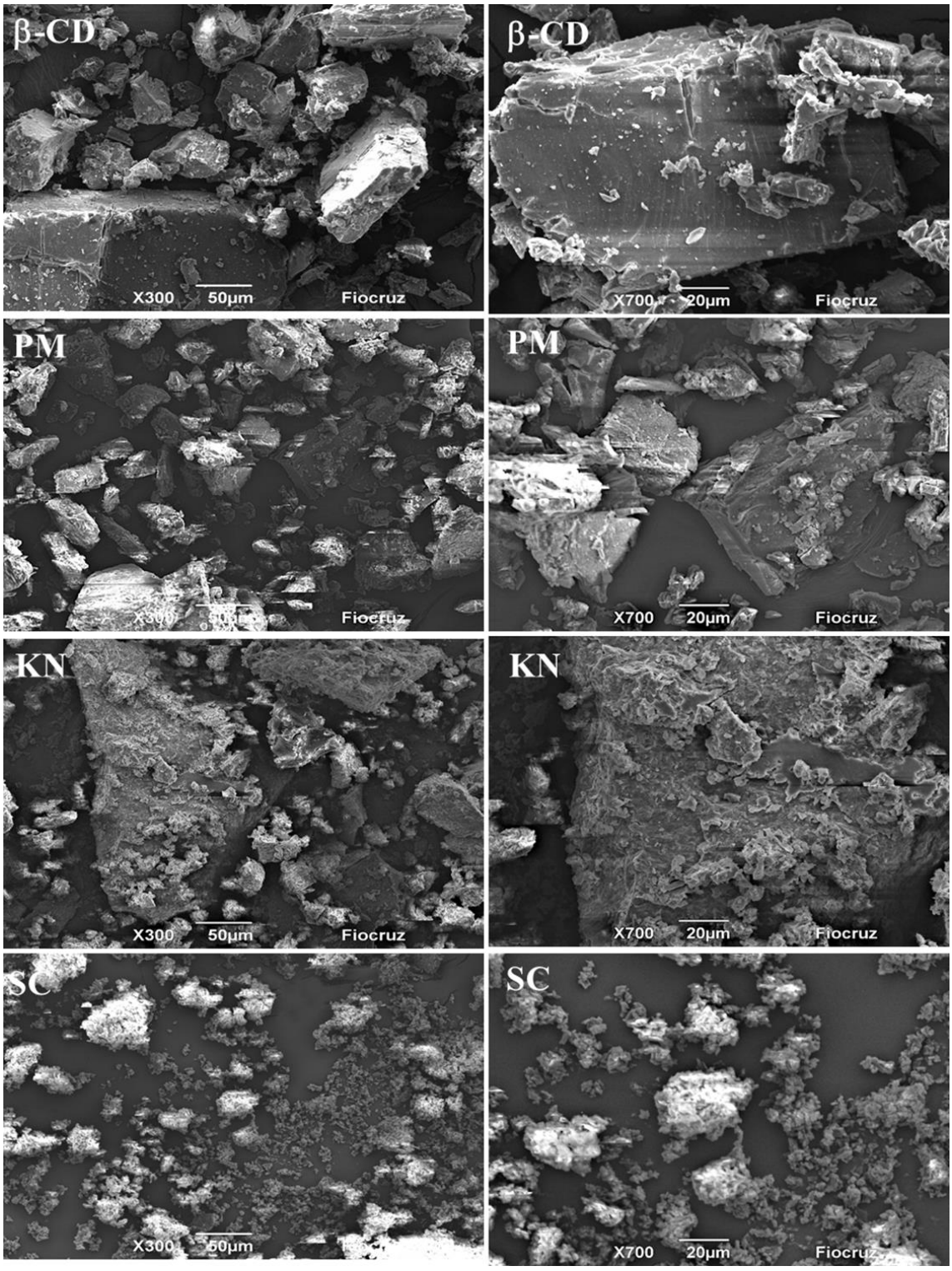

β-CD
X300 50µm Fiocruz
β-CD
X700 20µm Fiocruz
PM
X300 50µm Fiocruz
PM
X700 20µm Fiocruz
KN
X300 50µm Fiocruz
KN
X700 20µm Fiocruz
SC
X300 50µm
SC
X700 20µm Fiocruz

Figure 3.

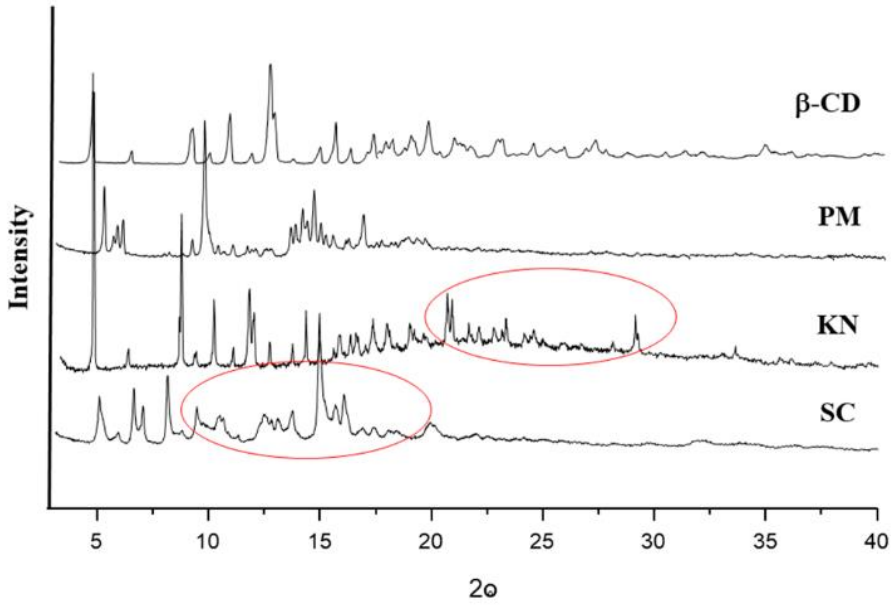


Figure 4.

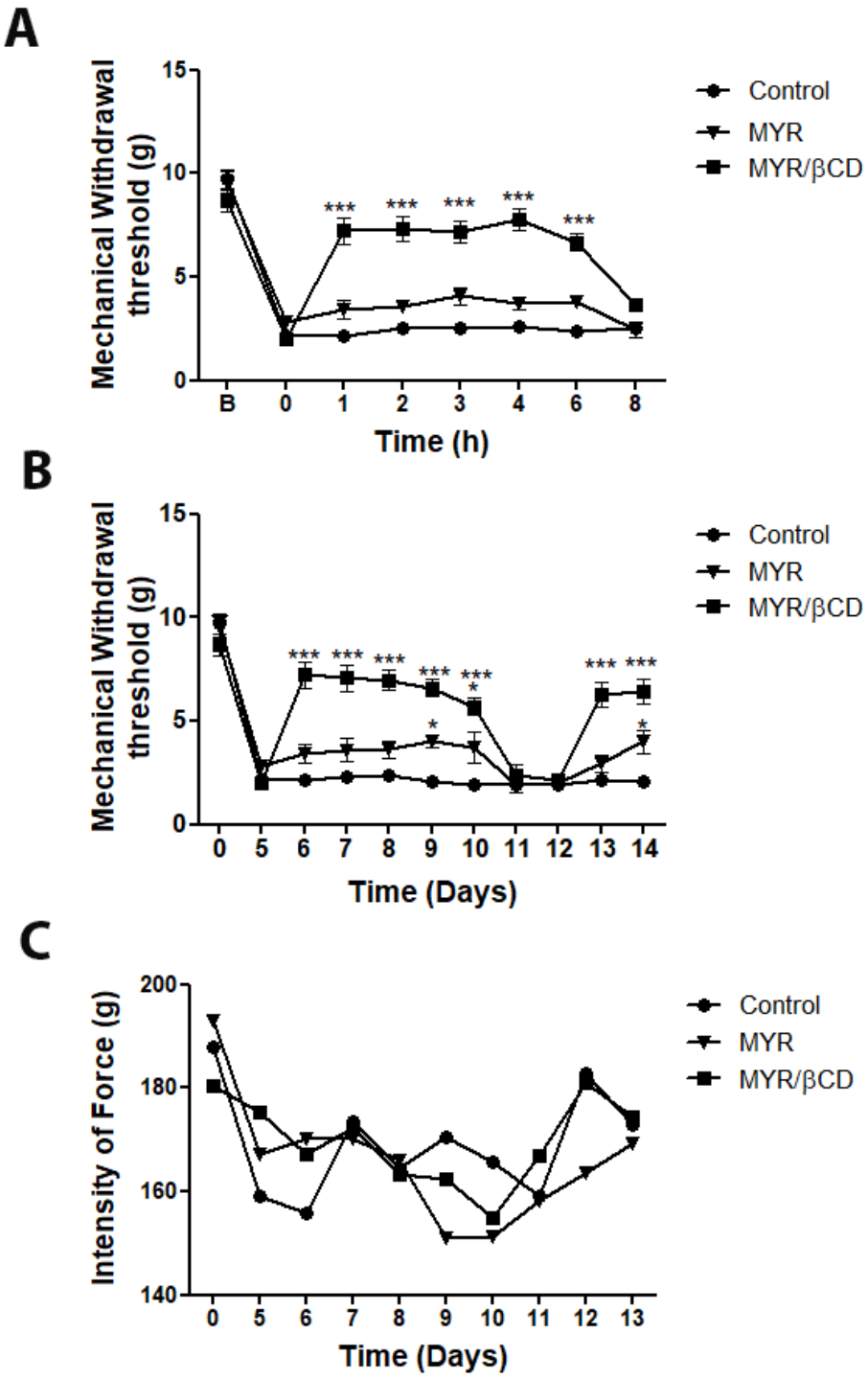

A
Mechanical Withdrawal threshold (g)
15
10
5
0
B
0
1
2
3
4
6
8
Time (h)
***
Control
MYR
MYR/βCD
B
Mechanical Withdrawal threshold (g)
0
5
6
7
8
9
10
11
12
13
14
Time (Days)
*
C
Intensity of Force (g)
200
180
160
140
0
5
6
7
8
9
10
11
12
13
Time (Days)

Figure 5.

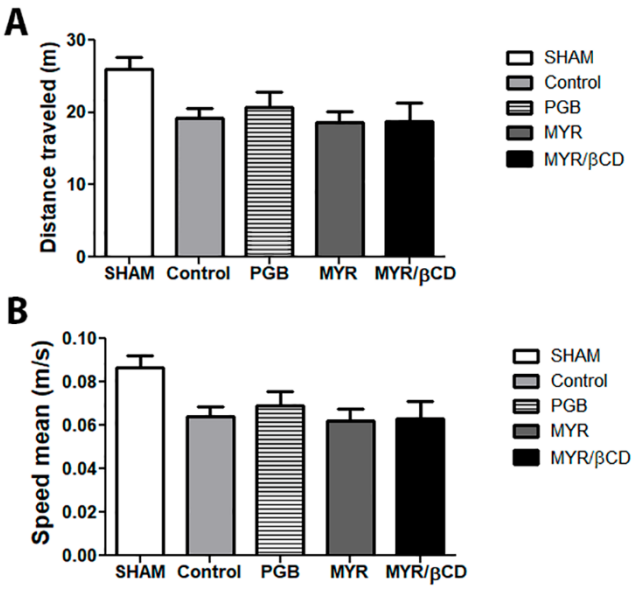

A
Distance traveled (m)
30
20
10
0
SHAM
Control
PGB
MYR
MYR/βCD
SHAM
Control
PGB
MYR
MYR/βCD
B
Speed mean (m/s)
0.10
0.08
0.06
0.04
0.02
0.00
SHAM
Control
PGB
MYR
MYR/βCD
SHAM
Control
PGB
MYR
MYR/βCD

Figure 6.

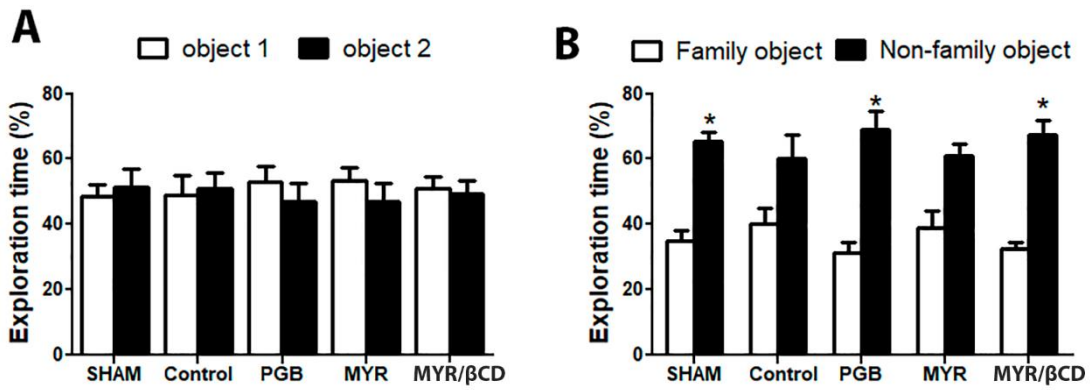

A
object 1
object 2
Exploration time (%)
80
60
40
20
0
SHAM
Control
PGB
MYR
MYR/βCD
B
Family object
Non-family object
Exploration time (%)
80
60
40
20
0
*
*
*
SHAM
Control
PGB
MYR
MYR/βCD

Figure 7.

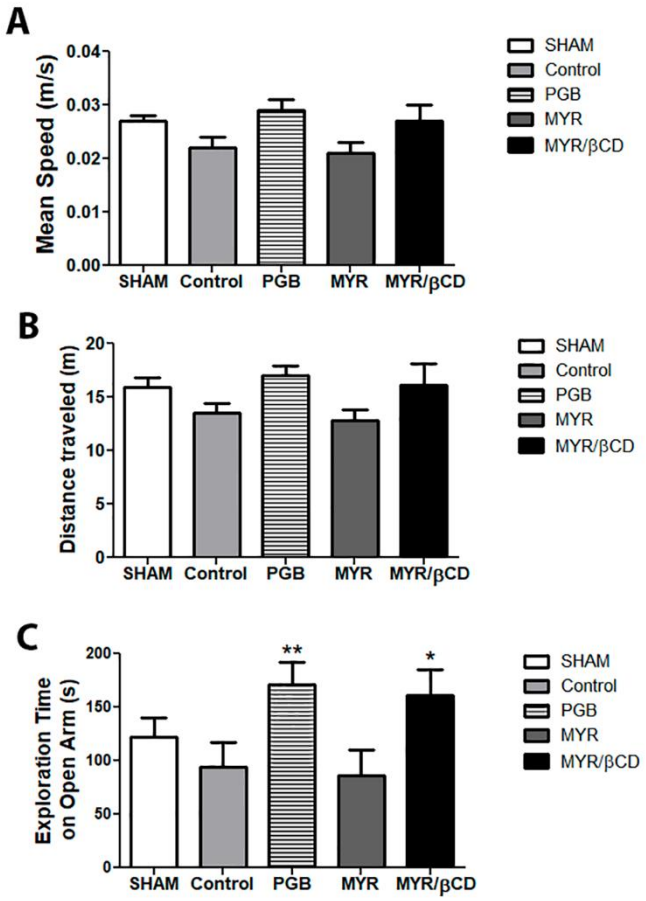
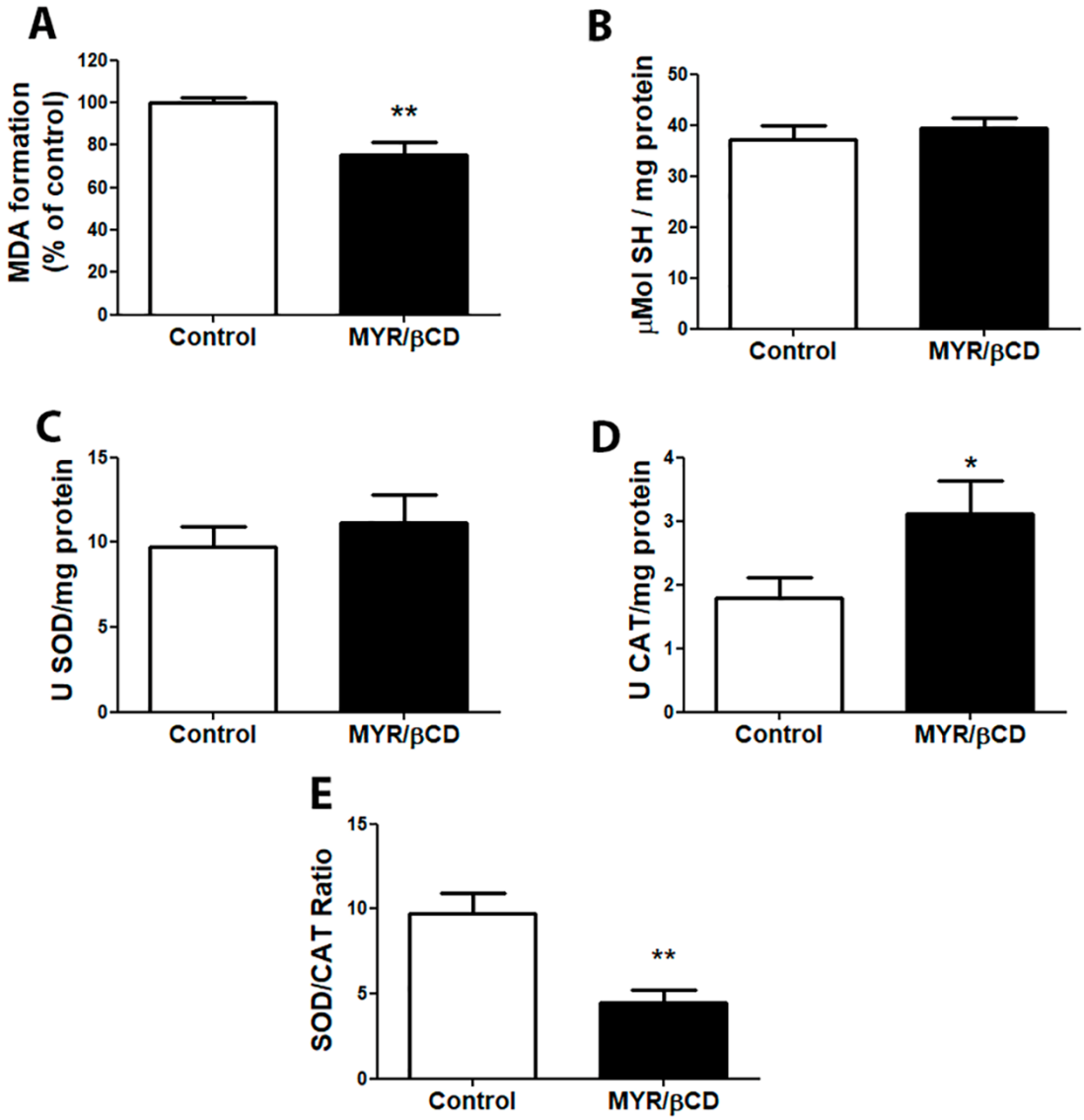
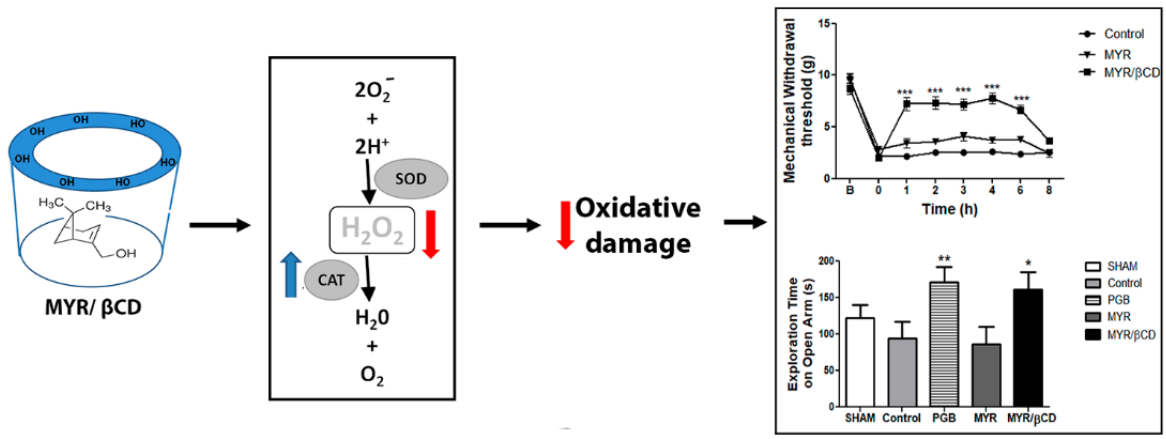

A
Mean Speed (m/s)
0.04
0.03
0.02
0.01
0.00
SHAM Control PGB MYR MYR/βCD
SHAM
Control
PGB
MYR
MYR/βCD
B
Distance traveled (m)
20
15
10
5
0
SHAM Control PGB MYR MYR/βCD
SHAM
Control
PGB
MYR
MYR/βCD
C
Exploration Time on Open Arm (s)
200
150
100
50
0
**
*
SHAM Control PGB MYR MYR/βCD
SHAM
Control
PGB
MYR
MYR/βCD

Figure 8.



Graphical abstract



UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COORDENAÇÃO DE PESQUISA
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

CERTIFICADO

Certificamos que a proposta intitulada "**Avaliação farmacológica de complexos de inclusão contendo mirtenol em β-ciclodextrina em modelo animal de hiperalgesia muscular não inflamatória** ", registrada com o **nº 16/2016**, sob a responsabilidade do **Prof. Dr. Lucindo José Quintans Júnior** que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Universidade Federal de Sergipe, em reunião de **14/03/2017**.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorizacão	Início: 01/07/2016, Término: 01/06/2018
Espécie/linhagem/raca	Camundongo Swiss
Nº de animais	64
Peso/Idade	25-30g / 2 meses
Sexo	M
Origem	Biotério Setorial da UFS.

Josemar Sena Batista

Prof. Dr. JOSEMAR SENA BATISTA
Coordenador do CEPA/UFS

Cidade Universitária "Prof. Aloísio de Campos"
Jardim Rosa Elze – São Cristóvão – SE
49100-000
Fones: 3212 6661/6606

UNIFESP
UNIVERSIDADE FEDERAL DE SÃO PAULO

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Avaliação de alterações emocionais e cognitivas do monoterpeno Mirtenol complexado em beta-Ciclodextrina no modelo experimental de Fibromialgia em camundongos", protocolada sob o CEUA nº 2888011216, sob a responsabilidade de **Alessandra Mussi Ribeiro** *e equipe; Francisca Rayanne da Silva Freitas; Klécia Santos dos Anjos; Lucindo José Quintans Jr.; Regina Helena da Silva; Sara Pereira da Silva* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de São Paulo (CEUA/UNIFESP) na reunião de 08/03/2017.

We certify that the proposal "Emotional and cognitive changes of the beta-cyclodextrin complex containing mirtenol on experimental model of fibromyalgia in mice.", utilizing Heterogenics mice, protocol number CEUA 2888011216, under the responsibility of **Alessandra Mussi Ribeiro** *and team; Francisca Rayanne da Silva Freitas; Klécia Santos dos Anjos; Lucindo José Quintans Jr.; Regina Helena da Silva; Sara Pereira da Silva* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Sao Paulo (CEUA/UNIFESP) in the meeting of 03/08/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 03/2017 a 05/2017 Área: Biociências

Origem:	CEDEME - Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia				
Espécie:	Camundongos heterogênicos	sexo: Machos	idade:	12 a 15 semanas	N: ---
Linhagem:	Swiss		Peso:	25 a 40 g	

Local do experimento: Para a realização do modelo de indução do dor muscular crônica os animais serão transferidos do biotério de manutenção para a sala de preparação/cirurgia anexa. Após os procedimentos retornarão para suas gaiolas moradia e para o biotério. Nos dias dos experimentos comportamentais os animais serão transferidos um a um para a sala de análise comportamental onde serão submetidos aos testes comportamentais e ao final serão eutanasiados.

São Paulo, 20 de março de 2018

Profa. Dra. Monica Levy Andersen
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de São Paulo

Dra. Tatiana Helfenstein
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de São Paulo

Normas da Revista

Oxidative Medicine and Cellular Longevity

Author Guidelines

Language Editing

Hindawi has partnered with Editage to provide an English-language editing service to authors prior to submission. Authors that wish to use this service will receive a 10% discount on all editing services provided by Editage. To find out more information or get a quote, please click here.

Submission

Manuscripts should be submitted by one of the authors of the manuscript through the online Manuscript Tracking System. Only electronic PDF (.pdf) or Word (.doc, .docx, .rtf) files can be submitted through the MTS, and there is no page limit. Submissions by anyone other than one of the authors will not be accepted. The submitting author takes responsibility for the manuscript during submission and peer review. If for some technical reason submission through the MTS is not possible, the author can contact omcl@hindawi.com for support.

Terms of Submission

Manuscripts must be submitted on the understanding that they have not been published elsewhere and are only being considered by this journal. The submitting author is responsible for ensuring that the article's publication has been approved by all the other coauthors. It is also the submitting author's responsibility to ensure that the article has all necessary institutional approvals. Only an acknowledgment from the editorial office officially establishes the date of receipt. Further correspondence and proofs will be sent to the author(s) before publication, unless otherwise indicated. It is a condition of submission that the authors permit editing of the manuscript for readability. All inquiries concerning the publication of accepted manuscripts should be addressed to omcl@hindawi.com. All submissions are bound by the Hindawi terms of service.

Peer Review

All manuscripts are subject to peer review and are expected to meet the standards of academic excellence. If approved by the editor, submissions will be considered by peer reviewers, whose identities will remain anonymous to the authors.

Our Research Integrity team will occasionally seek advice outside standard peer review, for example, on submissions with serious ethical, security, biosecurity, or societal implications. We may consult experts and the academic editor before deciding on appropriate actions,

including but not limited to: recruiting reviewers with specific expertise, assessment by additional editors, and declining to further consider a submission.

Concurrent Submissions

In order to ensure sufficient diversity within the authorship of the journal, authors will be limited to having two manuscripts under review at any point in time. If an author already has two manuscripts under review in the journal, they will need to wait until the review process of at least one of these manuscripts is complete before submitting another manuscript for consideration. This policy does not apply to Editorials or other non-peer reviewed manuscript types.

Article Processing Charges

The journal is Open Access. Article Processing Charges (APCs) allow the publisher to make articles immediately available online to anyone to read and reuse upon publication. For more details, please visit the Article Processing Charges information page.

Units of Measurement

Units of measurement should be presented simply and concisely using the International System of Units (SI).

Article Types

The journal will consider the following article types:

Research Articles

Research articles should present the results of an original research study. These manuscripts should describe how the research project was conducted and provide a thorough analysis of the results of the project. Systematic reviews may be submitted as research articles.

Clinical Studies

A clinical study presents the methodology and results of a study that was performed within a clinical setting. These studies include both clinical trials and retrospective analyses of a body of existing cases. In all cases, clinical studies should include a description of the patient group that was involved, along with a thorough explanation of the methodology used in the study and the results that were obtained.

When publishing clinical trials, Hindawi aims to comply with the recommendations of the International Committee of Medical Journal Editors (ICMJE) on trial registration. Therefore, authors are requested to register the clinical trial presented in the manuscript in a public trial registry and include the trial registration number at the end of the abstract. Trials initiated after July 1, 2005, must be registered prospectively before patient recruitment has begun. For trials initiated before July 1, 2005, the trial must be registered before submission.

Reviews

A review article provides an overview of the published literature in a particular subject area.

Formatting

An optional research article manuscript template can be downloaded here. We recommend that all manuscripts follow the structure below:

Title and Authorship Information

The following information should be included:

- Manuscript title
- Full author names
- Full institutional mailing addresses
- Email addresses

Abstract

The manuscript should contain an abstract. The abstract should be self-contained, citation-free, and should not exceed 200 words.

Introduction

This section should be succinct, with no subheadings.

Materials and Methods

This part should contain sufficient detail that would enable all procedures to be repeated. It can be divided into subsections if several methods are described.

Results and Discussion

This section may be divided into subsections or may be combined.

Main Text (Review only)

This section may be divided into subsections or may be combined.

Conclusions

This should clearly explain the main conclusions of the article, highlighting its importance and relevance.

Data Availability (excluding Review articles)

This statement should describe how readers can access the data supporting the conclusions of the study and clearly outline the reasons why unavailable data cannot be released. For guidance on composing a Data Availability statement, including template examples, please see here.

Conflicts of Interest

Authors must declare all relevant interests that could be perceived as conflicting. Authors should explain why each interest may represent a conflict. If no conflicts exist, the authors should state this. Submitting authors are responsible for coauthors declaring their interests.

Funding Statement

Authors must state how the research and publication of their article was funded, by naming financially supporting body(s) (written out in full) followed by associated grant number(s) in square brackets (if applicable), for example: “This work was supported by the Engineering and Physical Sciences Research Council [grant numbers xxxx, yyyy]; the National Science Foundation [grant number zzzz]; and a Leverhulme Trust Research Project Grant”.

If the research did not receive specific funding, but was performed as part of the employment of the authors, please name this employer. If the funder was involved in the manuscript writing, editing, approval, or decision to publish, please declare this.

Acknowledgments

All acknowledgments (if any) should be included at the very end of the manuscript before the references. Anyone who made a contribution to the research or manuscript, but who is not a listed author, should be acknowledged (with their permission).

References

Authors may submit their references in any style. If accepted, these will be reformatted in Chicago style by Hindawi. Authors are responsible for ensuring that the information in each reference is complete and accurate. All references should be numbered consecutively in the order of their first citation. Citations of references in the text should be identified using numbers in square brackets e.g., “as discussed by Smith [9]”; “as discussed elsewhere [9, 10]”. All references should be cited within the text and uncited references will be removed.

Preparation of Figures

Upon submission of an article, authors should include all figures and tables in the PDF file of the manuscript. Figures and tables should not be submitted in separate files. If the article is accepted, authors will be asked to provide the source files of the figures. Each figure should be supplied in a separate electronic file. All figures should be cited in the manuscript in a consecutive order. Figures should be supplied in either vector art formats (Illustrator, EPS, WMF, FreeHand, CorelDraw, PowerPoint, Excel, etc.) or bitmap formats (Photoshop, TIFF, GIF, JPEG, etc.). Bitmap images should be of 300 dpi resolution at least unless the resolution is intentionally set to a lower level for scientific reasons. If a bitmap image has labels, the image and labels should be embedded in separate layers.

Preparation of Tables

Tables should be cited consecutively in the text. Every table must have a descriptive title and if numerical measurements are given, the units should be included in the column heading. Vertical rules should not be used.

Supplementary Materials

Supplementary materials are the additional parts to a manuscript, such as audio files, video clips, or datasets that might be of interest to readers. Authors can submit one file of

supplementary material along with their manuscript through the Manuscript Tracking System. If there is more than one file, they can be uploaded as a .ZIP file.

A section titled “Supplementary Material” should be included before the references list with a concise description for each supplementary material file. Supplementary materials are not modified by our production team. Authors are responsible for providing the final supplementary materials files that will be published along with the article.

Proofs

Corrected proofs must be returned to the publisher within two to three days of receipt. The publisher will do everything possible to ensure prompt publication.

Copyright and Permissions

Authors retain the copyright of their manuscripts, and all Open Access articles are distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited.

The use of general descriptive names, trade names, trademarks, and so forth in this publication, even if not specifically identified, does not imply that these names are not protected by the relevant laws and regulations. The submitting author is responsible for securing any permissions needed for the reuse of copyrighted materials included in the manuscript.

While the advice and information in this journal are believed to be true and accurate on the date of its going to press, neither the authors, the editors, nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Conflicts of Interest

Conflicts of interest (COIs, also known as ‘competing interests’) occur when issues outside research could be reasonably perceived to affect the neutrality or objectivity of the work or its assessment. For more information, see our publication ethics policy. Authors must declare all potential interests – whether or not they actually had an influence – in a ‘Conflicts of Interest’ section, which should explain why the interest may be a conflict. If there are none, the authors should state “The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.” Submitting authors are responsible for coauthors declaring their interests. Declared conflicts of interest will be considered by the editor and reviewers and included in the published article.

Authors must declare current or recent funding (including for Article Processing Charges) and other payments, goods or services that might influence the work. All funding, whether a conflict or not, must be declared in the “Funding Statement”. The involvement of anyone other than the authors who 1) has an interest in the outcome of the work; 2) is affiliated to an organization with such an interest; or 3) was employed or paid by a funder, in the commissioning, conception, planning, design, conduct, or analysis of the work, the preparation or editing of the manuscript, or the decision to publish must be declared.

Ethical Guidelines

In any studies on human or animal subjects, the following ethical guidelines must be observed. For any experiments on humans, all work must be conducted in accordance with the Declaration of Helsinki (1964). Manuscripts describing experimental work which carries a risk of harm to human subjects must include a statement that the experiment was conducted with the human subjects' understanding and consent, as well as a statement that the responsible Ethical Committee has approved the experiments. In the case of any animal experiments, the authors must provide a full description of any anesthetic or surgical procedure used, as well as evidence that all possible steps were taken to avoid animal suffering at each stage of the experiment.